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Observations on the Effect of Cholesterol and Fat Supplementation on the Composition of Rabbit Liver and Plasma Lipides[1]

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Introduction

The extensive literature, recently summarized by Bronte-Stewart (1), on the effects of dietary fats on plasma cholesterol and plasma total lipides contains little quantitative information about the other lipide components of plasma or body tissues. Kritchevsky and associates (2, 3) have observed that rabbits receiving dienoic acid supplementation develop less severe aortal atheroma than those receiving supplementation of saturated acids. Mukherjee and co-workers (4) have reported the distribution of lipide components in rat plasma to be not significantly changed from normal after either a fat-free diet or a fat-free diet supplemented with linoleate. Okey and Harris (5) have claimed that fatty acids of rat liver phospholipides contain as much as 27% arachidonic acid. Klein (6) has reported the distribution of polyunsaturated fatty acids in sterol esters from livers and plasmas of rats maintained on five different dietary levels of linoleic acid. The predominant polyethenoid fatty acid of liver sterol esters was always linoleic, but plasma esters of animals receiving the highest level of dietary linoleic acid contained as much as 36% tetraenoic acid.

The present investigation, which must be regarded as a preliminary study, was undertaken to determine how the principal lipide components of liver and plasma are changed from normal in animals receiving cholesterol and fat supplements at two different levels of unsaturation.

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[2] One of the branches of Utilization Research, Agricultural Research Service, U. S. Department of Agriculture.

TABLE I

Distribution of Fatty Acids in Control and Experimental Diets

	Control		T + Ch[c]		O + Ch[c]	
	g/kg.	%[b]	*g./kg.*[a]	%[b]	*g./kg.*[a]	%[b]
Saturated	6.5	30.2	21.8	45.8	10.6	22.0
Monoene	2.8	13.1	13.3	27.5	10.0	20.5
Diene	10.1	46.5	10.3	21.6	25.2	52.1
Triene	1.7	8.0	1.9	4.0	2.1	4.3
Tetraene	0.3	1.6	0.4	0.8	0.4	0.8
Pentaene	0.03	0.15	0.04	0.1	0.05	0.1
Hexaene	0.1	0.6	0.1	0.2	0.1	0.2

[a] Grams fatty acids per kilogram of chow plus supplement.

[b] Per cent of individual fatty acids in the total fatty acids from chow plus supplement.

[c] T = tallow (beef); O = oil (corn); Ch = cholesterol.

Methods

In this study six normal adult male rabbits were divided into three groups of two animals each. Each received *ad libitum* a commercial chow[3] supplemented for the treated animals with 1.1% cholesterol and 3.3% of either beef tallow or corn oil. All animals were maintained on their respective diets for 3 months. They were then deprived of food overnight and sacrificed, and their tissues were stored at −20°C. until they were extracted. Liver tissue was extracted by 3:1 alcohol–ether and plasma by 4:1 methylal–methanol. In each case the extracted material was dissolved in petroleum ether and weighed after the evaporation of solvent. Total lipides from liver and plasma of individual animals were fractionated into their principal components by silicic acid column chromatography (7). After saponification of each component, the unsaponifiable material was removed and the liberated acids were quantitatively recovered. The fatty acids were isomerized and analyzed by ultraviolet spectrophotometry (8). Cholesterol was determined by the method of Sperry and Webb (9), and iodine numbers by the semimicro procedure of Luddy and coworkers (7).

Results and Discussion

The fatty acid composition of control and supplemented diets is given in Table I. The augmented diets contained about twice as much fat as the control diet. Supplemented diets differed principally in the saturated, monoenoic, and dienoic content.

After 3 months on their respective diets, all animals gained weight (Table II). Those receiving supplemented diets, particularly the tallow–cholesterol

[3] Composition of the commercial feed is as follows: crude protein (min.), 15%; crude fat (min.), 2.0%; crude fiber (max.), 1.8%; N.F.E. (min.), 46%.

Ingredients of the feed are listed as alfalfa meal, linseed oil meal, ground oats, ground yellow corn, cottonseed meal, soybean meal, molasses and mineral and vitamin supplements.

TABLE II

Effect of 3-Month Dietary Supplementation with Beef Tallow, Corn Oil and Cholesterol

	Control (av.)	T + Ch[c] (av.)	O + Ch[c] (av.)
Initial weight, *kg.*	3.3	3.0	3.5
Final weight, *kg.*	3.9	3.4	4.1
Liver total lipide[a]	5.74	11.69	8.05
Plasma total lipide[b]	581	2810	909

[a] Grams/100 g. wet liver.
[b] Milligrams/100 ml. plasma.
[c] T = tallow (beef); O = oil (corn); Ch = cholesterol.

TABLE III

Component Lipides of Liver and Plasma from Control and Experimental Rabbits

	Control (av.)		T + Ch[a] (av.)		O + Ch[a] (av.)	
	Liver	Plasma	Liver	Plasma	Liver	Plasma
	%	%	%	%	%	%
Hydrocarbon	0.3	0.9	0.0	0.4	0.8	0.2
Sterol ester	1.2	19.1	40.0	52.5	11.6	35.1
Glycerides	13.4	10.9	9.7	2.9	21.1	11.2
Free sterols	4.2	3.1	3.9	9.9	3.9	6.2
Free acids	2.5	3.4	5.8	6.4	6.1	2.7
Phospholipides	78.4	62.6	40.6	27.9	56.5	44.6

[a] T = tallow (beef); O = oil (corn); Ch = cholesterol.

supplement, had much greater concentrations of lipides in their plasma and liver than the control animals.

Table III shows the distribution of the principal components of liver and plasma lipides as obtained by silicic acid column chromatography. Within each dietary group the composition of liver lipides differed from plasma lipides, the per cent of phospholipides being higher and cholesteryl esters lower in the former than in the latter. The data show that the tallow–cholesterol supplemented diets produced the greatest increase of cholesteryl esters in both liver and plasma even though the same amount of fat was available for esterification in the oil–cholesterol fed group. Glycerides, though always present, were never the predominant lipide component. Free fatty acids in small amounts were present in both liver and plasma.

The effects of the dietary supplements on free and esterified cholesterol of the liver and plasma are presented in Table IV. The free cholesterol of liver remained constant at 4 mg./g. wet liver. Plasma-free cholesterol increased after dietary supplementation, but the ratio of free to total plasma

TABLE IV

Effect of Dietary Supplementation on the Free and Combined Cholesterol of Rabbit Liver and Plasma

	Control		T + Ch[a]		O + Ch[a]	
	Liver	Plasma	Liver	Plasma	Liver	Plasma
	mg./g. (wet)	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*
Combined cholesterol	0.74	66.2	27.9	885	6.9	244
Free cholesterol	4.15	18.0	3.9	279	3.9	71
Total cholesterol	4.9	84.2	31.8	1164	10.9	315

[a] T = tallow (beef); O = oil (corn); Ch = cholesterol.

cholesterol was essentially constant at 0.23 for the rabbit as compared to 0.27 in human serum as reported by Sperry (10). In every case, the free cholesterol obtained from silicic acid chromatography was weighed and confirmed by chemical analysis; combined cholesterol was calculated as 60% of the weight of the cholesteryl esters. Actual cholesterol analysis varied from 58.5 to 61.0%.

Table V shows the composition of the fatty acids of the cholesteryl esters isolated from the lipides of liver and plasma. The results are expressed in milligrams of fatty acids per gram of wet liver or per 100 ml. plasma. Although the tallow–cholesterol supplemented diet contained greater amounts of saturated acids than monoenoic acids, the liver of animals fed this diet contained greater amounts of the monoenoic acids. Perhaps the explanation is related to the capacity of the rabbit to convert saturated (but not polyunsaturated) acids to monoenoic acids, or the rabbit may utilize the saturated acids preferentially for energy. There was also an increase of

TABLE V

Fatty Acids in the Cholesteryl Esters of Liver and Plasma

	Control		T + Ch[a]		O + Ch[a]	
	Liver	Plasma	Liver	Plasma	Liver	Plasma
	mg./g. (wet)	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*
Saturated	<0.3[b]	5.0	1.2	175	1.7	27
Monoenoic	<0.3[b]	20.2	15.5	416	1.5	110
Dienoic	0.08	25.8	2.4	147	0.9	55
Trienoic	0.02	1.0	0.4	17	0.1	4
Tetraenoic	0.03	1.8	0.2	4	0.1	3
Pentaenoic	0.01	0.1	0.1	1	0.0	0
Hexaenoic	0.0	0.0	0.0	0	0.0	0

[a] T = tallow (beef); O = oil (corn); Ch = cholesterol.

[b] The quantity of cholesteryl esters recovered from control liver was too small for determination of the specific amounts of saturated and monoenoic acids.

TABLE VI

Fatty Acids in Phosphatides of Liver and Plasma

	Control		T + Ch[a]		O + Ch[a]	
	Liver	Plasma	Liver	Plasma	Liver	Plasma
	mg./g. (wet)	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*
Saturated	9.7	52.3	8.8	179	9.7	73.4
Monoenoic	2.1	24.7	5.9	74.2	2.3	43.0
Dienoic	5.7	15.3	5.9	85.5	7.3	34.3
Trienoic	0.2	0.8	0.3	5.1	0.2	1.8
Tetraenoic	1.9	3.9	1.6	16.7	1.9	6.6
Pentaenoic	0.3	1.0	0.4	4.3	0.3	2.0
Hexaenoic	0.2	0.0	0.1	0.0	0.1	0.0

[a] T = tallow (beef); O = oil (corn); Ch = cholesterol.

other individual fatty acids in the cholesteryl esters of liver and plasma, particularly in the rabbits receiving tallow–cholesterol supplementation.

The amounts of the individual fatty acids in the phospholipides of liver and plasma are shown in Table VI. Liver individual fatty acids remained approximately constant in all animals, but plasma fatty acids were increased by dietary supplementation. In each column of Table VI, the summed weights of the unsaturated fatty acids approximately equaled that of the saturated acids. This evidence supports the concept that the typical phospholipide molecule contains one saturated and one unsaturated fatty acid. It was surprising that the plasma dienoic and tetraenoic acids of the rabbits receiving tallow–cholesterol supplement were many times greater than the control values, and over twice those from the oil-supplemented animals.

Table VII shows that monoenoic acid was the most variable component of the liver and plasma fatty acids from total lipides. In each column, if

TABLE VII

Fatty Acid Composition of Liver and Plasma Total Lipide

	Control		T + Ch[a]		O + Ch[a]	
	Liver	Plasma	Liver	Plasma	Liver	Plasma
	mg./g. (wet)	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*	*mg./g. (wet)*	*mg./100 ml.*
Saturated	12.3	91	14.8	361	16.9	121
Monoenoic	3.9	62	26.3	492	7.8	163
Dienoic	7.5	54	10.0	232	12.4	101
Trienoic	0.4	3	0.8	22	0.3	6
Tetraenoic	2.0	6	2.0	21	2.2	10
Pentaenoic	0.5	1	0.4	5	0.3	2
Hexaenoic	0.2	0	0.1	0	0.1	0

[a] T = tallow (beef); O = oil (corn); Ch = cholesterol.

the amount of monoenoic acid was subtracted from the summed weights of all the individual fatty acids, the resulting quantity represented the total of the less variable fatty acid components. The percentage of each individual acid of the less variable group is relatively constant.

Other findings, not included in the tables, were made in these experiments. Both rabbits receiving tallow–cholesterol exhibited atheromatous lipide in their aortic arches as did also one animal receiving corn oil. Analyses showed that the depot fat of rabbits receiving corn oil and cholesterol, as would be expected was more unsaturated than that of normal controls, whereas the depot fat of rabbits receiving tallow was less unsaturated. The fatty acid composition of the liver and plasma glycerides obtained in the silicic acid column separation was also determined. The composition was found similar to the depot fat from the same animal.

Summary

1. A study has been made of liver and plasma lipides from rabbits fed commercial chow, chow supplemented with beef tallow and cholesterol, and chow plus corn oil and cholesterol.

2. Animals fed the supplemented diets showed substantial increases in liver and plasma total lipides, particularly in cholesteryl esters; those receiving tallow and cholesterol supplements showed the greatest increases.

3. Monoenoic acid was the principal fatty acid of the liver and plasma cholesteryl esters from rabbits on augmented diets. This was particularly true in animals receiving tallow and cholesterol supplementation.

4. The fatty acid composition of the liver phospholipides was relatively uniform, whereas that of the plasma showed large increases in each fatty acid in the supplemented groups. The amounts of dienoic and tetraenoic acids were greatest in the plasma phospholipides of the tallow–cholesterol supplemented animals.

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